

The University of Chicago

HODGKIN'S DISEASE

A DISSERTATION SUBMITTED TO THE
FACULTY OF THE DIVISION OF THE BIO-
LOGICAL SCIENCES IN CANDIDACY FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF PATHOLOGY

1933

By

PAUL E. STEINER, M.D., PH.D.

Fellow in Medicine of the National Research Council

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Reprinted from
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HODGKIN'S DISEASE

SEARCH FOR AN INFECTIVE AGENT AND ATTEMPTS AT EXPERIMENTAL REPRODUCTION

PAUL E. STEINER, M.D., PH.D.

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Since the report by Sternberg¹ in 1898 claiming an etiologic relationship between tuberculosis and Hodgkin's disease a voluminous and conflicting mass of evidence has accumulated. This evidence has been discussed in recent reviews by Wallhauser,² Simonds,³ Vasiliu⁴ and Chevallier and Bernard.⁵

Interest in the possible tuberculous origin of Hodgkin's disease was rearoused by the reports of L'Esperance,⁶ who stated that she was able to produce tuberculosis in chickens and in previously vaccinated guinea-pigs by injection of material from persons with Hodgkin's disease. When the studies to be reported in this publication were begun there were no contradictory or confirmatory reports available. Since then Utz and Keatinge⁷ stated that they have confirmatory evidence, which they, however, fail to give. They have already begun the treatment of Hodgkin's disease with chicken serum prepared by injection of material from tissues showing Hodgkin's disease, and they have reported encouraging results in thirty patients so treated.⁸

Branch⁹ stated that certain investigators failed to confirm the experimental results of L'Esperance. Of these, Van Es investigated three cases and Aronson two cases. Schulz, on the other hand, isolated acid-fast organisms, apparently of avian type, from a node in one case. More recently van Rooyen¹⁰ reported that he obtained negative results in six cases studied by cultural methods and by inoculation and grafting

From the Department of Pathology of the University of Chicago.

1. Sternberg, C.: *Ztschr. f. Heilk.* **19**:21, 1898.
2. Wallhauser, A.: *Arch. Path.* **16**:522 and 672, 1933.
3. Simonds, J. P.: *Arch. Path.* **1**:394, 1926.
4. Vasiliu, T.: *Ann. d'anat. path.* **8**:815, 1931.
5. Chevallier, P., and Bernard, J.: *La maladie de Hodgkin*, Paris, Masson & Cie, 1932.
6. L'Esperance, E.: (a) *J. Immunol.* **16**:37, 1929; (b) **18**:127 and 133, 1930; (c) *Ann. Surg.* **93**:162, 1931.
7. Utz, L., and Keatinge, L.: *M. J. Australia* **1**:397, 1931.
8. Utz, L., and Keatinge, L.: *M. J. Australia* **1**:521, 1932.
9. Branch, A.: *Arch. Path.* **12**:253, 1931.
10. van Rooyen, C. E.: *Brit. M. J.* **1**:50, 1933.

into fowls. Garrod¹¹ in one case was unable to produce tuberculosis in pigeons. Wallhauser² and H. L. Stewart¹² were unable to find evidence of tuberculosis in chickens into which the diseased tissues had been injected.

F. W. Stewart and Doan¹³ recently presented immunologic evidence suggesting a relationship to avian tuberculosis. They found that the precipitin titer with tuberculophosphatide in known cases of Hodgkin's disease was within the range observed in known cases of tuberculosis, and that the titer with avian tuberculophosphatide tended to be higher than that with human tuberculophosphatide. These results were not obtained with other types of lymphadenopathies.

The present experiments were designed (1) to test the diseased tissues in a series of fifteen cases of Hodgkin's disease (as well as in eight control cases of different lymphomas) for ability to produce tuberculosis in chickens, guinea-pigs, rabbits, dogs and mice, and (2) to test these same tissues by special cultural methods for the presence of tubercle bacilli. In each case of Hodgkin's disease the diagnosis had been established by examination of microscopic sections of the diseased tissues by two or more experienced, disinterested pathologists, who used as their criterion the classic description by Reed.¹⁴ Lack of space precludes a description of each case in detail. Most of the patients were followed clinically. In the evolution of their disease and in their symptoms, physical signs, response to roentgen therapy and other clinical manifestations they presented typical Hodgkin's disease. Because of the scope of the investigation, the significant but negative results obtained and the objections raised by certain critics to the procedures used by other investigators, the experimental procedures will be given in detail.

EVIDENCE FROM INOCULATIONS AND TRANSPLANTATIONS OF THE DISEASED TISSUES INTO ANIMALS

Experiments were planned to determine the transmissibility of possible infectious agents, whether bacteria or virus, and the transplantability of tissues which may possibly be regarded as neoplastic.

Material from 15 persons with proved Hodgkin's disease was inoculated into 35 chickens, 24 guinea-pigs, 18 rabbits, 7 dogs and 3 mice. Tissues secured at autopsy from suspicious lesions in these animals were in turn used in further attempts at transmission or passage of the disease in 4 chickens, 4 guinea-pigs and 4 rabbits.

11. Garrod, L. P., in *Rose Research on Lymphadenoma*, Bristol, John Wright & Sons, Ltd., 1932, p. 105.

12. Stewart, H. L.: *J. Lab. & Clin. Med.* **18**:281, 1932.

13. Stewart, F. W., and Doan, C. A.: *Ann. Surg.* **93**:141, 1931.

14. Reed, D. M.: *Johns Hopkins Hosp. Rep.* **10**:133, 1902.

Similarly conducted control experiments were made in 8 cases of different types of lymphadenopathy. These included 3 cases of lymphosarcoma (1 of reticulum cell type and 2 of lymphocytic cell type), 3 cases of leukemia (1 each of aleukemic myelosis, aleukemic lymphadenosis and chronic lymphatic leukemia) and 2 cases of tuberculous lymphadenitis. These cases were chosen because they were indistinguishable from cases of Hodgkin's disease by their clinical features or by their blood pictures. The biopsy or the subsequent course determined the diagnosis. Inoculations were made into 22 chickens, 15 guinea-pigs, 14 rabbits, 1 dog and 9 mice. Tissues from some of these animals in turn were used in attempted passage of the disease in 7 chickens, 6 guinea-pigs and 6 rabbits. Thus material in 23 cases was injected into 199 animals of different species.

A second type of control consisted of 23 chickens that were not subjected to injections. This group was chosen to provide a check on the occurrence of spontaneous tuberculosis—reputedly common in this species—and on environmental factors. Representative specimens were selected from successive lots of chickens purchased. They were kept in cages alternating with those of the fowl given injections and were subjected to the same environmental conditions.

Experimental Procedure.—The lymphogranulomatous and control tissues were secured aseptically by biopsy or at autopsy. A representative portion of each lymph node or spleen used for injection was prepared for histologic examination. The remainder was chopped and ground in a sterile mortar with sterile physiologic solution of sodium chloride. When the tissue was fibrous the maceration was aided by the use of sterile sand. The suspension was injected as soon as possible, in some instances within two hours after the removal of the tissue. Intravenous injections were made through a 23 gage hypodermic needle. Subcutaneous, intraperitoneal and intracerebral injections were made through a needle of 20 gage or larger. An attempt was made to inject particles that would barely pass through the needles. On the whole rather large doses were used (tables 1 and 2).

Partly grown chickens of both sexes were used. At first they were purchased in the open market. After a tuberculous chicken had been found, all were subsequently purchased directly from a farm. The chickens had been raised in brooders on an open range and were shipped to the laboratory in crates previously unused. In addition, they were tuberculin-tested and kept away from known sources of accidental infection. The conditions of food and environment were not ideal. Certain chickens showed signs of mineral and vitamin deficiency and should have been readily susceptible to infection. After receiving injections they were kept in cages 3 tiers high. Thus there was opportunity for infection of those in the bottom rows with material dropped from those above. The quarters were rather dark, no direct sunlight being admitted. There was little opportunity for exercise. Every chicken received an injection in the wing vein from the suspension described. It was found that despite an occasional immediate death from embolism chickens tolerate coarse suspensions better than do rabbits. In addition to the intravenous injections some chickens were given subcutaneous and intraperitoneal injections.

TABLE 1.—*Experiments in Which Human Tissues Affected with Hodgkin's Disease Were Injected into Animals.*

Case	Sex of Patient	Age	Source of Material	Kind of Material	Amount, Gm.	Injections				Animals That Became Tuberculous	Reinjections				Animals That Became Tuberculous
						Guinea-Pigs	Rabbits	Chickens	Dogs	Mice	Diseased Chickens	Guinea-Pigs	Rabbits	Chickens	
1	M	13	Biopsy	Axillary	2.4	2	1	2	..	..		..	..	..	None
2	M	52	Autopsy	Retropertoneal	10.0	2	2	2	..	..	O 4	2	2	2	None
4	M	8	Autopsy	Retropertoneal	10.0	3	3	3	..	..	Chick 18	2	2	2	All except 1 rabbit
5	M	27	Biopsy	Cervical, axillary	2.0	3	2	3	..	..		..	..	..	None
7	M	30	Biopsy	Cervical	1.0	..	..	3	..	..		..	..	..	None
8	F	24	Biopsy	Cervical	0.5	..	..	2	..	..		..	..	..	None
10	M	14	Biopsy	Cervical		2	2	3	..	..		..	..	..	None
12	M	10	Autopsy	Splenic, tracheal, retro-peritoneal	10.0	2	2	3	1	..		..	..	..	None
13	M	28	Biopsy	Cervical	2.0	2	2	3	1	..		..	..	..	None
14	M	30	Biopsy	Cervical	0.5	..	..	3	1	3		..	..	..	None
15	F	19	Biopsy	Axillary	1.0	..	2	2	1	..		..	..	..	None
17	M	21	Biopsy	Cervical	0.5	2	..	2	..	..		..	..	..	Guinea-pig 47
21	F	34	Biopsy	Axillary	3.5	2	2	2	..	..		..	..	..	None
22	M	37	Biopsy	Cervical	0.6	2	..	..	2	..		..	..	..	None
23	F	24	Biopsy	Cervical	1.0	2	..	2	1	..		..	..	..	None
Total (15 cases).....						24	18	35	7	3		4	4	4	

TABLE 2.—Control Experiments

Sex of Case Patient	Age	Source of Material	Kind of Material	Amount, Gm.	Injections				Animals That Became Tuberculous	Reinjections				Animals That Became Tuberculous	
					Guinea- Pigs	Rab- bits	Chick- ens	Dogs		Mice	Diseased Chicken	Guinea- Pigs	Rab- bits		Chick- ens
Tuberculous adenitis															
11	M	20	Cervical	4.0	2	2	3	..	2	2 guinea-pigs, 2 mice	2	2	2	2	None
16	F	66	Cervical	0.5	..	..	2	..	..	None		..	..	..	..
Lymphosarcoma															
3	M	50	Retroperi- toneal	10.0	3	2	3	..	..	1 guinea-pig	C13 C15	2 2	2 2	2 2	None None
9	M	40	Splenic, retro- peritoneal		2	2	3	1	3	None		..	..	..	..
19	F	79	Retroperi- toneal	11.0	2	2	3	..	4	None		..	..	..	..
Leukemia															
6	M	27	Splenic, peri- aotic		2	2	3	..	..	None		..	..	..	..
18	F	51	Cervical	1.0	2	2	2	..	..	None		..	..	..	..
20	M	72	Retroperi- toneal		2	2	3	..	..	None		..	..	..	..
Controls (no injections given).....					..	..	23	..	..	None		..	..	..	..
Total (8 cases).....					15	14	45	1	9		6	6	6	6	6

The rabbits and guinea-pigs received the usual laboratory care. They were kept in hygienic surroundings away from tuberculous animals. Young animals were used and they gained weight rapidly. They were also tuberculin-tested before use. The rabbits were given intraperitoneal injections. In addition, some were given subcutaneous injections. The guinea-pigs usually received subcutaneous injections, but some also received intraperitoneal injections. Transplantation of thin wafers of tissue was made subcutaneously and intraperitoneally into some guinea-pigs and rabbits.

Each dog received an intracerebral injection through a small perforation of the skull in the frontal region. Since tuberculous dogs do not give a cutaneous reaction¹⁵ to tuberculin this test was often omitted. The intracerebral injections were made because of the success reported by Feldman¹⁶ in infecting dogs with avian tubercle bacilli by this method.

After the injections had been given, all animals were watched carefully for signs of disease. They were weighed frequently. Tuberculin tests were performed frequently on the chickens, both avian and human tuberculins of proved potency being used. Old tuberculin 50 per cent of full strength was used in 0.1 cc. doses. At the termination of the experiment some chickens had received seven different tests with each tuberculin. There was no evidence that any of them had been sensitized by the tuberculin of previous tests. The rabbits and guinea-pigs were tuberculin-tested less frequently, but all were tested before the end of the experiment. For these tests, decreasing dilutions of human and avian tuberculins down to that of 1:10 were used.

The animals that did not spontaneously succumb to disease were killed by overanesthetization between the ninth and thirteenth months. The dogs were an exception in that these experiments were terminated between one hundred and two hundred and seventy-four days. All animals were carefully examined grossly at autopsy for evidence of disease. Tissues were then preserved in fixing solution and sections were examined as a routine from lungs, liver, spleen and kidneys as well as from other organs as indicated. All tissues that were tuberculous or that contained lesions even remotely suggesting a granuloma were stained for acid-fast organisms. For this purpose, the Cooper¹⁷ modification of the Ziehl-Neelsen stain was most satisfactory.

Certain animals at autopsy presented lesions the nature of which was uncertain. The tissues showing these lesions were subjected to further study by three methods: 1. Direct smears were stained by the Ziehl-Neelsen method. 2. Cultures were made according to the method described for growing the tubercle bacillus. 3. Passage experiments were carried out (tables 1 and 2) in which these tissues were used for injection into other animals, the methods being used which were employed with the original material.

Results.—The results of the experiments with tissues from persons with Hodgkin's disease are given in table 1; those of the experiments with the control materials, in table 2, and a summary, in table 3. Several experiments deserve special comment.

Of the 35 chickens that received injections of suspensions of tissues from persons with Hodgkin's disease and 22 that received injections

15. Steiner, P. E.: *Am. Rev. Tuberc.*, to be published.

16. Feldman, W. H.: *Am. J. Path.* **7**:147, 1931.

17. Cooper, F. B.: *Arch. Path.* **2**:382, 1926.

of suspensions of control tissues, only 1 died of undisputable tuberculosis. In this experiment 3 guinea-pigs, 3 rabbits and 3 chickens received injections of the same retroperitoneal lymph node suspension. Of the 9 inoculated animals, only 1 chicken acquired tuberculous lesions. The patient, a boy 8 years of age, died two years after the onset of symptoms referable to Hodgkin's disease (case 4). One month after injection of the material the tuberculin reaction in this chicken (C 18) was strongly positive. The fowl lost over half of its original weight and died, nine weeks after inoculation, with a massive tuberculous infection of the liver, spleen and lungs. On histologic examination the acid-fast bacilli were present in great numbers. Unfortunately, spontaneous

TABLE 3.—*Summary: Transplantations and Injections into Animals;
222 Experiments*

Diagnosis of Condition of Patients from Whom Tissues Were Taken for Injection into Animals	No. of Cases	Guinea-Pigs		Rabbits		Chickens		Dogs		Mice	
		Injections	Tuberculous	Injections	Tuberculous	Injections	Tuberculous	Injections	Tuberculous	Injections	Tuberculous
Hodgkin's disease.....	15	24	1	18	0	35	1†	7	0	3	0
Reinjections (passage).....	(2)	4	..	4	..	4					
Injected controls:											
Lymphosarcoma.....	3	7	1	6	0	9	0	1	0	7	0
Reinjections (passage).....	(3)	4	..	4	..	5					
Leukemia.....	3	6	0	6	0	8	0				
Tuberculous adenitis.....	2	2	2	2	0	5	0	..	..	2	2
Reinjections (passage).....	(1)	2	..	2	..	2					
Controls not given injections.....	..	..	..	..	..	23*					
Total.....	23	49	4	42	0	91	1	8	0	12	2

* These controls were not given injections but were kept in cages alternating with those of the chickens given injections and were subjected to the same environmental conditions.

† One of the chickens given injections.

tuberculosis cannot be ruled out. This chicken was one of the first in the series and was not tuberculin-tested prior to the injection of the suspension of tissue from the patient with Hodgkin's disease. A suspension of the liver and spleen of this chicken was injected into 2 chickens, 2 rabbits and 2 guinea-pigs. One chicken died with tuberculosis, principally of the liver, in six weeks, and the other, of tuberculosis of the liver, spleen, lungs and bone marrow in nine weeks. One of the guinea-pigs died in six weeks with marked generalized tuberculosis. The second guinea-pig acquired a positive reaction to avian tuberculin but failed to react to human tuberculin; when killed ten months after injection it had only a few scars in the lungs, which on histologic examination could not be definitely identified as tuberculous. Of the 2 rabbits, one died two weeks after receiving the injection, during a minor epidemic of enteritis, and revealed no sign of tuberculosis, while the other died

in six weeks of extensive tuberculosis. Cultures of the original chicken liver and spleen on various mediums yielded acid-fast bacilli which on injection into chickens killed them with typical tuberculosis.

Many other chickens died during the course of the experiments. Perhaps the most common cause of death was leukemia or a leukemoid reaction. Such chickens did not acquire a positive reaction to tuberculin, although occasionally a slight edematous swelling occurred at the test site. They did not lose much weight, and at autopsy the gross observations were not those of typical tuberculosis. Histologic examination revealed the nature of the disease. There was an absence of stainable acid-fast bacteria in such lesions, cultures for acid-fast organisms were negative and in several passage experiments no tuberculosis was produced. Furthermore such leukemoid reactions occurred in the control groups of chickens, both those given and those not given injections.

Another lesion worthy of special mention which was encountered 4 times was a granuloma of the wall of the gastro-intestinal tract, usually in the cecum. These masses grossly resembled one described by L'Esperance^{6a} as tuberculous. Microscopically, however, they showed varying amounts of new granulation tissue and fibrous connective tissue. There were areas of massive necrosis and also smaller necrotic foci, at the periphery of which there was sometimes a marked giant cell reaction. The chickens producing the granulomas lost much weight. They had persistently negative reactions to tuberculin. No acid-fast bacteria were ever seen in multiple stained sections and no growth of acid-fast organisms was recovered in cultures. Passage experiments failed to produce tuberculosis in the various species of animals used. Two chickens in which such tumors developed were *controls not given injections*; another was a control given an injection of a suspension of lymphosarcomatous tissue, and the fourth was a chicken used in a passage experiment in which a suspension of tissue from a leukemic chicken was injected. These lesions are therefore considered to be nontuberculous.

Lesions of still another type were encountered in 3 chickens. These consisted of firm, grayish-yellow, discrete but unencapsulated nodules in the liver, measuring from 4 to 10 mm. in diameter. On histologic examination the nodules were seen to be made up of many types of cells, among which small and large mononuclear cells predominated. There were numerous cells resembling myelocytes, as well as some large mononuclear giant cells. Cells with a granular, eosinophilic cytoplasm were numerous. Cells of the type of the plasma cell were also present. All these cells were supported in a reticular framework which resembled that of the original liver (fig. 1). In addition to these features common to all, one nodule showed several focal accumulations of densely packed cells with small round and oval nuclei with an even chromatin pattern and a very eosinophilic, finely granular, moderately abundant cytoplasm.

Immediately surrounding these foci were found rings of endothelioid giant cells with clusters of pale nuclei and indefinitely outlined cytoplasm (fig. 2).

As far as one can determine from an examination of photomicrographs, these lesions show a remarkable resemblance to those illustrated by L'Esperance.^{6a} However, I do not consider them tuberculous for the following reasons: No acid-fast organisms were demonstrable in them. The chickens on repeated tests never gave a positive reaction to tuberculin. Moreover, two of the lesions occurred in *controls that had not had injections*. The third was found in an apparently healthy

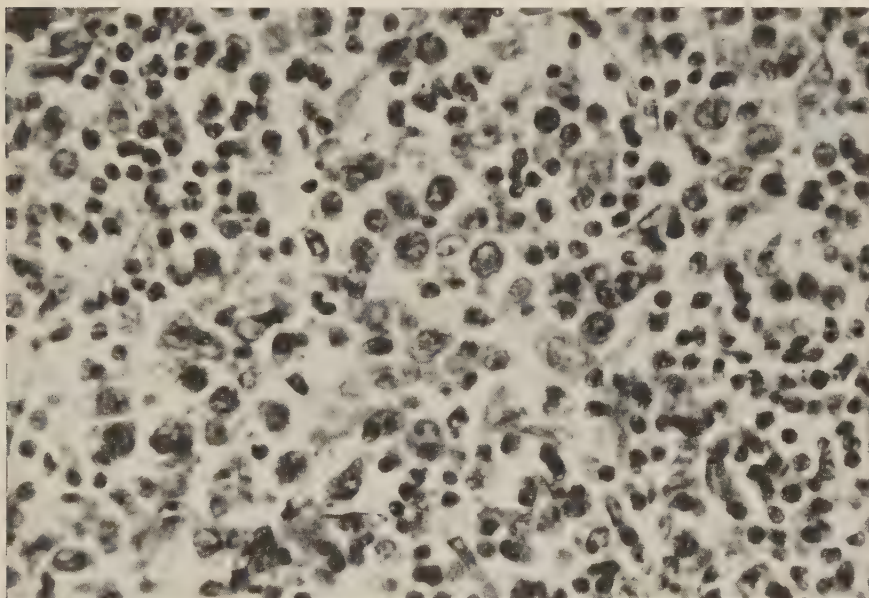


Fig. 1.—High power photomicrograph showing the types of cells found in the hepatic nodules. $\times 1450$.

chicken killed nine months after it had received an injection of a suspension of tissue from the patient in case 13, one of Hodgkin's disease. The other 2 chickens given injections of the same material and examined post mortem at the same time showed no disease. None of the lesions of this type showed any tendency to dissemination or to production of a fatal disease. Unfortunately, no passage experiments were performed with these lesions.

The other chickens that died before the termination of the experimental period showed a variety of lesions. They failed to show positive reactions to tuberculin or evidence of tuberculosis. Five chickens that received injections of suspensions of tissue from 2 persons with tuber-

culous adenitis presented no sign of tuberculosis. Likewise those given injections of tissues from persons with leukemia and lymphosarcoma did not become tuberculous.

Of the 24 guinea-pigs into which injections or transplantations of Hodgkin's tissues were made, only 1 contracted tuberculosis. This animal (G-P47) presented enlargement of the inguinal lymph nodes on the side of the injection and when tested four months later had a positive reaction to human tuberculin and a negative reaction to avian tuberculin. The original lymph node obtained for biopsy from the cervical region of a man 21 years of age showed no microscopic evidence

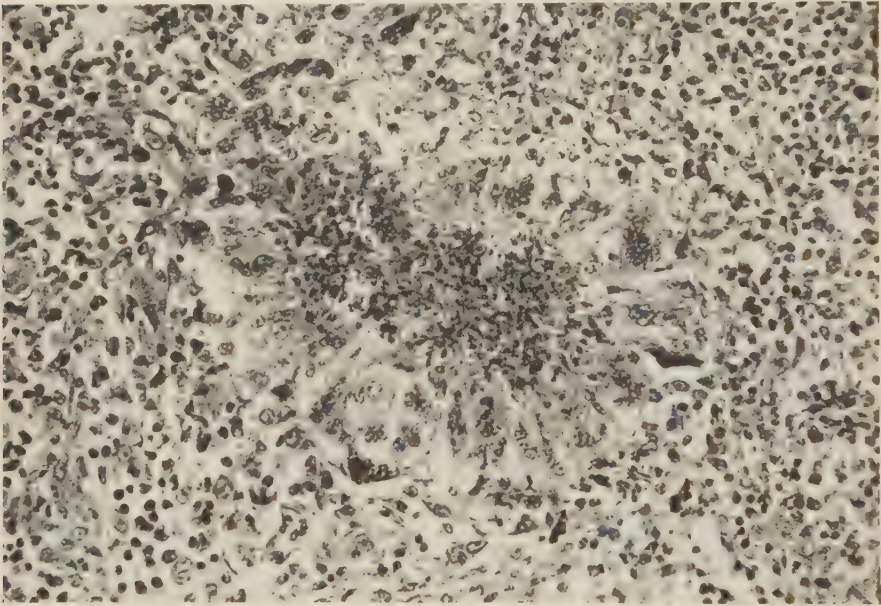


Fig. 2.—Type of lesion, resembling a tubercle, found in the hepatic nodule shown in figure 1. This is considered nontuberculous. $\times 500$.

of tuberculosis (case 17). The animal died six and one half months after it received the injection, with extensive tuberculosis of the lungs, liver, spleen and lymph nodes. The other guinea-pig and the 2 chickens that received injections of the same material never showed positive reactions to tuberculin and at the termination of the experiment showed no evidence of tuberculosis.

Two guinea-pigs that received injections of a suspension of tissue from one of the control patients with tuberculous adenitis died of tuberculosis. Of 13 guinea-pigs that received injections of material from 3 persons with lymphosarcoma and 3 with leukemia, 1 developed tuberculosis (case 3). This animal (G-P17) died of marked generalized

tuberculosis five months after tissue from a retroperitoneal lymph node had been engrafted into it. The node showed the histologic picture of a reticulum cell type of lymphosarcoma. No special significance was attributed to this observation since the injection of nontuberculous lymph node tissue from this location not infrequently produces tuberculosis in animals.

No rabbit, not even the 2 given injections of the material from the 2 patients with tuberculosis, contracted tuberculosis during the experiments. Seven dogs received intracerebral injections of suspensions of tissue from persons with Hodgkin's disease and one, of a suspension of lymphosarcomatous tissue, without showing evidence of tuberculosis. Of the mice that became tuberculous during the experiments, both had received injections of material from a person with tuberculous adenitis.

Because of the success of Nungester¹⁸ in enhancing the virulence of bacteria by suspending them in sterile gastric mucin, this method was tried in 1 case of Hodgkin's disease. One dog and 2 guinea-pigs failed to acquire tuberculosis after being given injections of the diseased tissue suspended in mucin.

EVIDENCE FROM CULTURES

Cultural studies were undertaken, not primarily to determine the bacterial flora of the tissues from the patients with Hodgkin's disease, but to demonstrate acid-fast organisms if they were present. Many of the reported bacteriologic studies on lymphogranulomatous tissues antedate the development of cultural methods and procedures which today are highly recommended for the isolation of acid-fast bacteria. Thus the cultural studies served as a control of the injections and permitted application of some of the reputedly superior methods for the isolation of tubercle bacilli.

An attempt was also made to repeat the observations of Busni.¹⁹ She reported that from tissues showing Hodgkin's disease she was able regularly to grow bacteria which, when examined at from twelve to twenty-four hours, were acid-fast and resembled Koch's bacillus. After another period of from twelve to twenty-four hours they appeared as small nonacid-fast cocci. After further incubation, at a time when macroscopic growth was not yet evident, they resembled masses of staphylococci. Special procedures were devised to make analogous observations on cultures concerned here.

Experimental Procedure.—Fragments of diseased spleen and lymph node tissues were inoculated into culture mediums as soon as possible after they were obtained for biopsy or at autopsy. This was usually within a few hours after their removal

18. Nungester, W. J.: Proc. Soc. Exper. Biol. & Med. **30**:120, 1932.

19. Busni, N.: Virchows Arch. f. path. Anat. **268**:614, 1928.

Thin, wafer-like slices or macerated tissues were transferred to the surface of solid mediums in culture tubes. In some cases contamination was suspected and the tissues were treated with 6 per cent sulphuric acid, 5 per cent ovalic acid or 3 per cent acetic acid for thirty minutes or more before inoculation. The mediums used were Sweany's²⁰ veal, cream, milk, egg, medium, Corper's potato²¹ and Herrold's egg yolk medium.²² The latter was modified in a few instances by the addition of 5 per cent glycerin. Loeffler's serum, blood agar and Petroff's egg medium were also used at various times. Numerous tubes were inoculated with material from each case. Incubation was continued for periods up to nine months.

It is generally recognized that partial carbon dioxide tensions are superior for the isolation of some acid-fast bacteria. Accordingly some of the culture tubes were incubated in 10 per cent carbon dioxide tensions at 37 C. Since lower temperatures have been advocated as advantageous for certain strains, some tubes were kept at room temperature. The criteria for sterility have been modified by the pioneer work of Hauduroy²³ on serial plate transfers. With certain specimens that appeared sterile after prolonged incubation his technic was employed for periods up to twenty days before the tubes were discarded as sterile. In an attempt to repeat the observations of Busni, examinations of apparently sterile cultures were made at twenty-four hour intervals by grasping the implanted tissue in the culture tubes with sterile forceps, touching the surface which was in contact with the culture medium to the surface of a sterile glass slide, and then examining the slide preparations after staining them by the Ziehl-Neelsen and Gram methods.

Results.—No cultures of definitely acid-fast bacilli were obtained by these methods from tissues in 11 cases of Hodgkin's disease. Although the mediums were not especially suitable for the growth of the diphtheroids so frequently recovered by many investigators, bacteria of this type were grown in 4 cases, and other common bacteria usually considered to be contaminants were grown occasionally. In 2 cases the culture tubes remained sterile.

In only 2 instances were observations made resembling those reported by Busni. From a cervical lymph node removed for biopsy in a case of Hodgkin's lymphogranulomatosis, implanted on modified Herrold medium and incubated in 10 per cent carbon dioxide tension, small nonacid-fast bacilli of medium size appeared on the impression slide preparations. Observation of these organisms revealed that most of the body was gram-negative but that it contained from one to four, usually two, gram-positive granules of a diameter equal to that of the bacillus. The organisms containing four granules appeared to be short chains of streptococci. When only two granules were present the appearance suggested a diplococcus. After another forty-eight hours of incubation rather small, slightly beaded bacilli resembling diphtheroids, but with a tendency to be acid-fast, were numerous. Macroscopic evidence

20. Sweany, H. C., and Evanoff, M.: *Am. Rev. Tuberc.* **18**:661, 1928.

21. Corper, H. J.: *J. A. M. A.* **91**:371, 1928.

22. Herrold, R. D.: *J. Infect. Dis.* **48**:236, 1931.

23. Hauduroy: *Compt. rend. Soc. de biol.* **97**:1392, 1927.

of growth first appeared on the ninth day, and an organism resembling a diphtheroid, without any tendency to be acid-fast, slowly grew out.

In another case the sterile slide impressions prepared after twenty-four hours of incubation of tissue secured for biopsy from a cervical lymph node in a case of Hodgkin's disease showed clusters of gram-positive coccus-like organisms. These, however, failed to become grossly visible, and although the rapid serial plate technic was used, no bacterial colonies were ever obtained.

Five controls consisting of tissues from 3 patients with lymphosarcoma and 2 with leukemia yielded no significant organisms. No acid-fast bacteria were grown from them. Diphtheroids were grown in 1 case and staphylococci were grown in 2.

COMMENT

In this intensive search for tubercle bacilli in tissues from patients with Hodgkin's disease by inoculation of the tissues into a variety of species of animals and by cultural methods little evidence of such organisms was found. No acid-fast strains resembling tubercle bacilli were grown by cultural methods, and inoculation of material from fifteen patients with Hodgkin's disease into animals gave tuberculosis in only 1 guinea-pig and 1 chicken. In the latter animal a spontaneous tuberculous infection was not ruled out. In neither of these cases were other animals infected by injections of the same material.

If the results of Dolgopol²⁴ showing that chickens are insusceptible to infection by the human type of tubercle bacillus are accepted—and this observation is confirmed in the 2 control cases of tuberculous adenitis used in this series—it becomes apparent that the 1 chicken that contracted tuberculosis in these experiments was infected with tubercle bacilli probably of avian type. This is substantiated by the results of injection of infected tissues and of cultures of acid-fast bacilli obtained from this chicken into other chickens.

One important factor in the propagation of tuberculosis in animals is the size of the infecting dose. This was borne in mind throughout the investigation. Consequently, in many experiments, large amounts of the diseased tissues were macerated and thick suspensions were prepared. Of these, large amounts were injected (see table 1). Nevertheless the incidence of infection remained low.

Another important factor is the resistance of the test animals. Overcrowding, poor ventilation, dark quarters, undernourishment and vitamin deficiencies lead to a higher incidence of infection. Among my experimental animals the chickens alone were subjected to some of these adverse conditions. Still they failed to present tuberculosis. This leads to the belief that acid-fast organisms pathogenic for chickens were

24. Dolgopol, V. B.: *J. Infect. Dis.* **49**:216, 1931.

extremely infrequent in, or entirely absent from, the tissues of the patients with typical Hodgkin's disease whom I studied.

Ewing²⁵ emphasized the factor of duration of time in the production of tuberculosis with such tissues. Although many of my experiments were allowed to continue for over one year there was no increased incidence of infection.

The incidence of tuberculous infection in guinea-pigs in this series was also smaller than that reported by some investigators. One guinea-pig of the 24 given injections of the tissues showing Hodgkin's disease was infected. This incidence closely approaches that reported by Twort²⁶ and by Gordon.²⁷ The latter produced tuberculosis in 3 of 40 guinea-pigs subjected to injection.

The diphtheroids so frequently grown in cultures of tissues from persons with Hodgkin's disease who have not been treated become increasingly difficult to recover from tissues irradiated by roentgen rays. It is not known whether the destruction by irradiation also applies to the tubercle bacillus. To avoid this possibility, many tissues that had never been irradiated by roentgen rays were used. This apparently had no influence on the final result.

During the examination of smears from diseased tissues stained by the Ziehl-Neelsen method a phenomenon was noticed which at first was confusing. Acid-fast granules and debris suggestive of degenerated bodies of tubercle bacilli were often seen. In sections of these same tissues stained by the Cooper method the nature of the acid-fast materials became apparent. A restudy of the original smears then supported the opinion that they were either granules from eosinophilic leukocytes or debris resulting from the destruction of red blood cells. The cytoplasm of the latter was found to be unusually tenacious for the fuchsin stain.

SUMMARY

Diseased splenic and lymph node tissues from 15 patients with Hodgkin's disease (lymphogranulomatosis) together with control diseased tissues from 8 patients with other lymphomas were investigated (1) for the ability of the diseased tissue to produce tuberculosis in chickens, guinea-pigs, rabbits, dogs and mice, (2) for the disease-producing capacity of these tissues as grafts, and (3) for the presence of tubercle bacilli or atypical acid-fast bacteria in these same tissues, special cultural methods being used. The diseased tissues from the 23 patients were

25. Ewing, J.: *Neoplastic Diseases*, ed. 3, Philadelphia, W. B. Saunders Company, 1928, p. 407.

26. Twort, C. C.: *J. Path. & Bact.* **33**:539, 1930.

27. Gordon, M. H., in *Rose Research on Lymphadenoma*, Bristol, John Wright & Sons, Ltd., 1932.

injected or transplanted into 199 animals of the species mentioned. An additional group of 23 chickens that were not given injections served as a control of environmental factors, especially of the occurrence of spontaneous tuberculosis. A group of 8 dogs received intracerebral injections. The animals were allowed to survive for periods varying from nine to thirteen months. The criteria used for the diagnosis of tuberculosis in the animals following injection were: (1) the acquisition of a positive reaction to tuberculin, (2) the gross and microscopic morphologic structure of tuberculous lesions, (3) the presence of stainable acid-fast bacilli in suspicious lesions, (4) the growth of acid-fast bacilli from such lesions on culture mediums and (5) the production of tuberculosis in animals in passage experiments.

In the entire group of animals given injections of diseased human tissues, tuberculosis occurred in 1 chicken following injection of material from a patient with Hodgkin's disease, 1 guinea-pig following injection of tissue from a patient with Hodgkin's disease, and 1 guinea-pig following injections of lymphosarcomatous tissue. In the infected chicken a spontaneous infection was not ruled out. In passage experiments with tissues from these infected animals tuberculosis was again produced.

Numerous lesions in diseased chickens superficially suggesting tuberculosis were considered nontuberculous because they did not definitely satisfy *any* of the five criteria previously laid down.

No evidence was found that the diseased human tissues were transplantable or that they were capable of inducing in animals lesions with a similar histologic structure.

No strains of acid-fast bacteria were grown from these diseased human tissues by modern cultural methods. Likewise the occurrence of acid-fast forms of bacteria reported to exist as a transient phenomenon early in the cultures of these tissues was not confirmed.

Avian tubercle bacilli detectable by the methods used were apparently not present in the tissues of the 15 patients with Hodgkin's disease.

